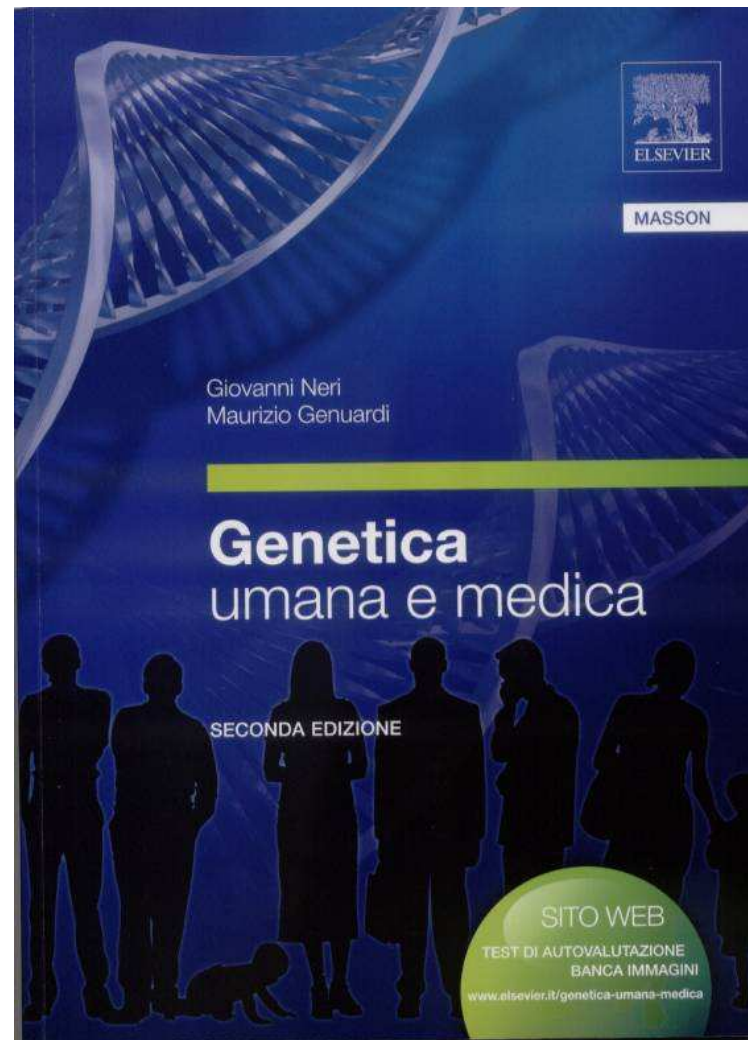
CORSO INTEGRATO DI GENETICA 2011-2012

Corso di Laurea Magistrale in Medicina e Chirurgia
II anno di corso, I semestre
Aula C Istituti Biomedici
Martedi e Giovedi 10.15-1.45, Mercoledi 8.30-10
4.10 - 21.12.2011

Discipline e Docenti:

Genetica Molecolare: Elisabetta Trabetti, Cristina Bombieri

Genetica Umana: Pier Franco Pignatti, Alberto Turco, Giovanni Malerba

Testo consigliato

Orario Ricevimento Studenti

Prof PF Pignatti: mercoledi ore 15-16
Prof A Turco: mercoledi ore 16-17
Dott.ssa E Trabetti: mercoledi ore 13-14
Dott.ssa C Bombieri: mercoledi ore 14-15
Dott. G Malerba: martedì ore 16-17

Materiale didattico

I file PDF con le presentazioni verranno messi nel sito dopo ogni lezione

Sito web: http://medgen.univr.it/didattica/

Selezionare: Genetica A.A. 2011/2012

La distribuzione in internet di questo materiale è intesa per uso strettamente personale. Qualsiasi altro uso (riproduzione in copie multiple, allestimento e commercializzazione di dispense) è espressamente vietato

CORSO INTEGRATO DI GENETICA

A.A.2011/2012

Prof Alberto Turco

4.10.11

Lezioni 1 e 2

CITOGENETICA GENERALE

(Neri-Genuardi cap.4)

Un po' di storia………….

1956: IDENTIFICAZIONE DEL NUMERO CORRETTO DI CROMOSOMI NELL'UOMO

Tjio JH and Levan A (1956). The chromosome number of man. *Hereditas*, **42**, 1–6. Reproduced with permission from the Mendelian Society of Lund.

THE CHROMOSOME NUMBER OF MAN

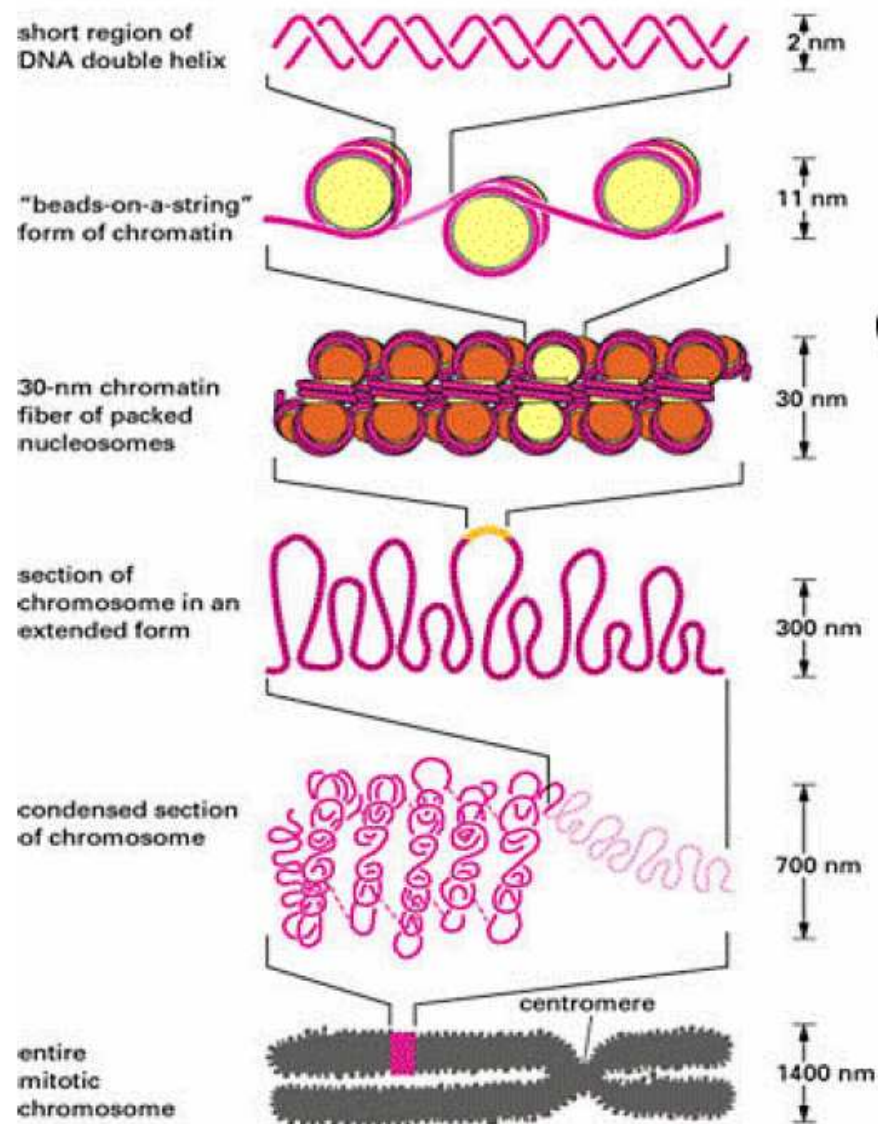
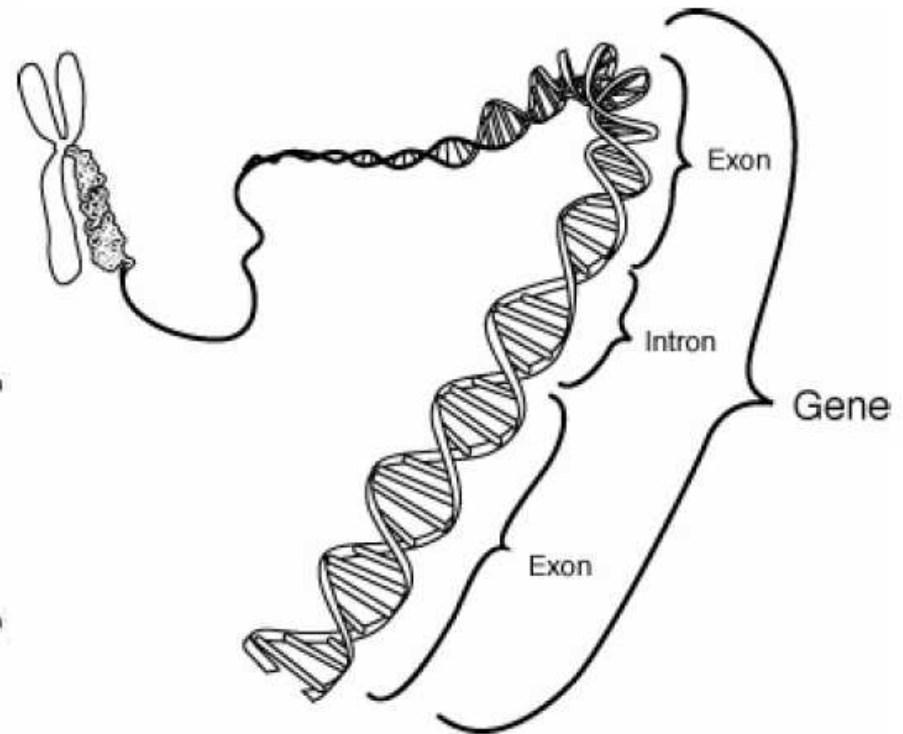
By *JOE HIN TJIO* and *ALBERT LEVAN*

ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME LABORATORY, INSTITUTE OF GENETICS, LUND, SWEDEN

WHILE staying last summer at the Sloan-Kettering Institute, New York, one of us tried out some modifications of HSU's technique (1952) on various human tissue cultures carried in serial *in vitro* cultivation at that institute. The results were promising inasmuch as some fairly satisfactory chromosome analyses were obtained in cultures both of tissues of normal origin and of tumours (LEVAN, 1956).

Later on both authors, working in cooperation at Lund, have tried still further to improve the technique. We had access to tissue cultures of human embryonic lung fibroblasts, grown in bovine amniotic fluid; these were very kindly supplied to us by Dr. RUNE GRUBB of the Virus Laboratory, Institute of Bacteriology, Lund. All cultures were primary explants taken from human embryos obtained after legal abortions. The embryos were 10—25 cm in length. The chromosomes were studied a few days after the *in vitro* explantation had been made.

In our opinion the hypotonic pre-treatment introduced by HSU, al-

Avvolgimento cromatina

Cromosoma = ordine più alto di organizzazione cromatina

PREPARAZIONE CARIOTIPO STANDARD
(Citogenetica convenzionale)

-Cc somatiche nucleate (es.leucociti sangue periferico, fibroblasti)

-Mitogeno (PHA fitoemoagglutinina)

-Colchicina (blocco in metafase)

-Soluzione ipotonica

-Fissazione

-Colorazione (bandeggio)

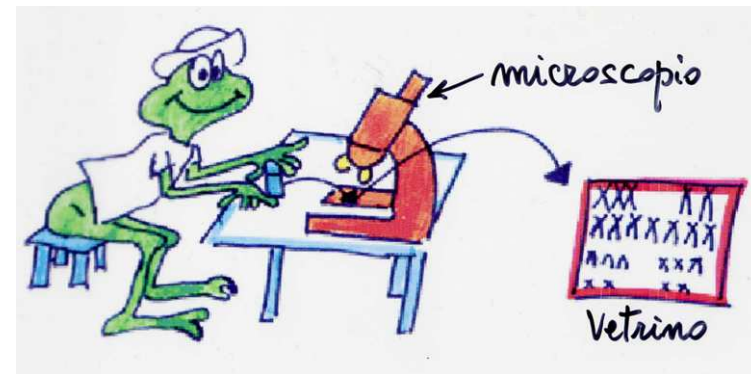



Tabella 4.1 Caratteristiche e specificità dei tipi di regioni cromosomiche identificate dalle tecniche di bandeggio

	Q/G-positive	R-positive	C-positive	NOR-positive
Localizzazione	Braccia corte e lunghe	Braccia corte e lunghe	Regioni pericentromeriche e braccio lungo del cromosoma Y	"Satelliti" (braccia corte) dei cromosomi acrocentrici
Induttori chimici	Quinacrina/tripsina	5-bromodesossiuridina + denaturazione fotolitica e termica	Idrato di bario	Nitrato di argento
Contenuto in geni	Basso	Alto (geni in singola copia)	Nessuno	Geni ripetuti in tandem per le subunità 5S e 28S dell'RNA ribosomiale
Replicazione del DNA	Tardiva	Precoce	Tardiva	–
Attività trascrizionale	Bassa	Elevata	Assente	Alta

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

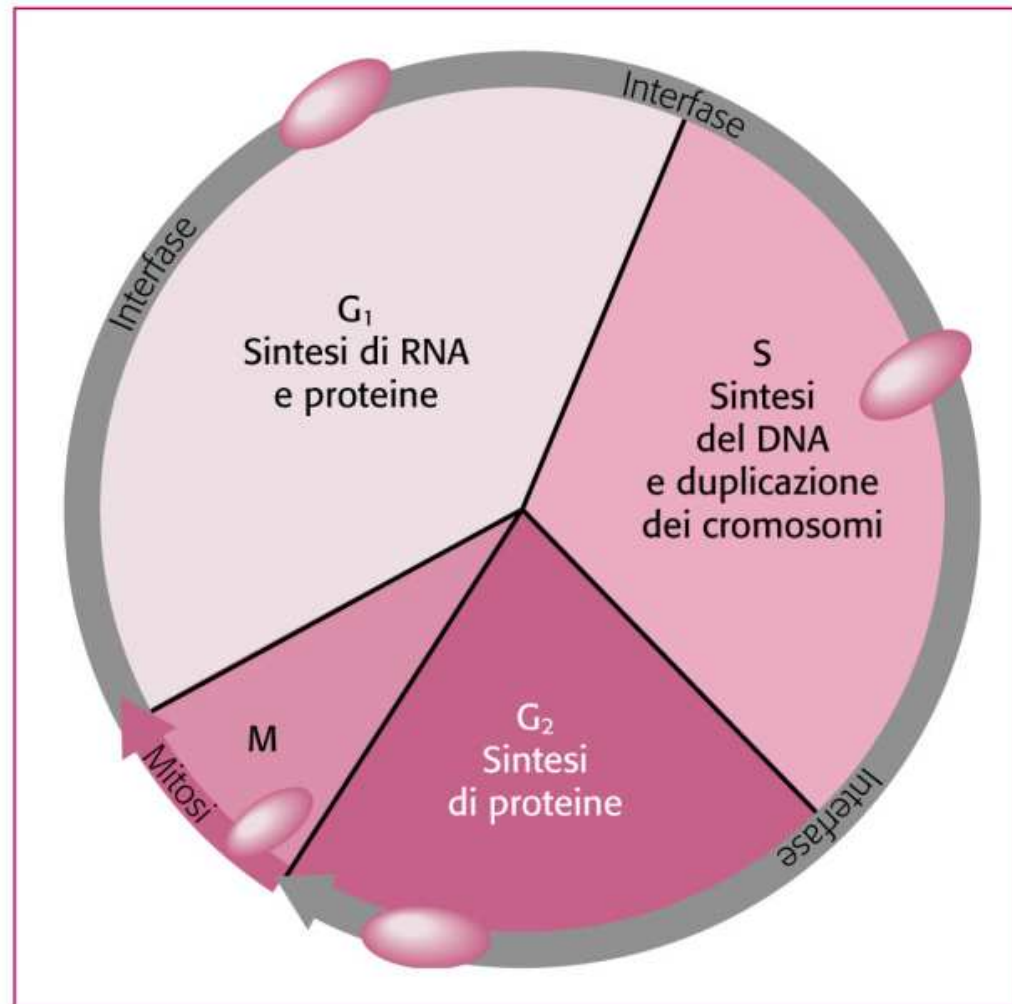



Figura 4.14 Rappresentazione schematica del ciclo cellulare. Il ciclo cellulare è un'ordinata serie di eventi che determinano la crescita della cellula e la sua divisione in due cellule figlie. Le cellule che non si dividono entrano in una fase di quiescenza (G_0), finché non rientrano nel ciclo cellulare, le cui fasi sono G_1 (sintesi di RNA e proteine), S (sintesi del DNA), G_2 (preparazione alla mitosi) e M (mitosi). La mitosi si divide a sua volta in quattro fasi.

NORMAL MALE
46,XY
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

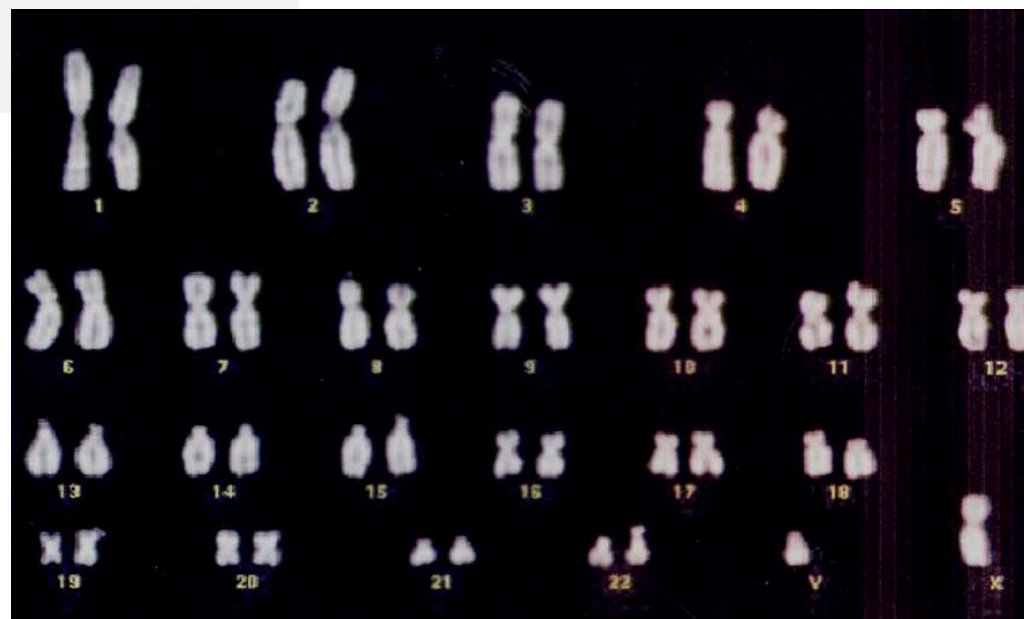
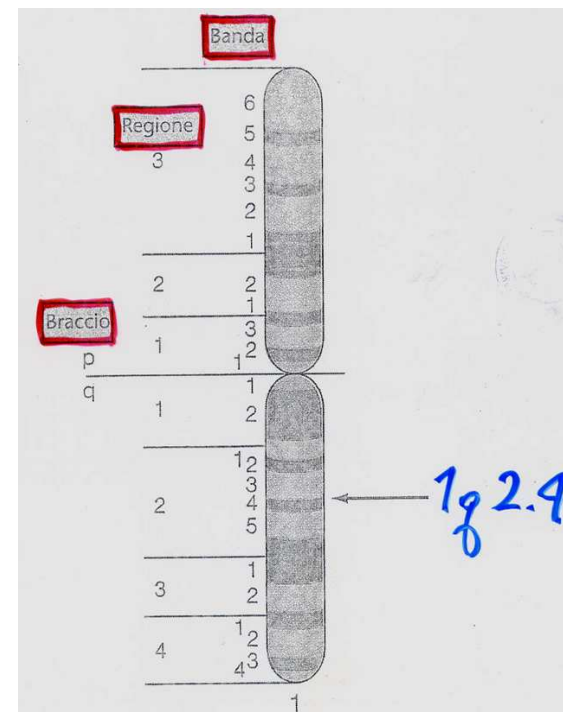
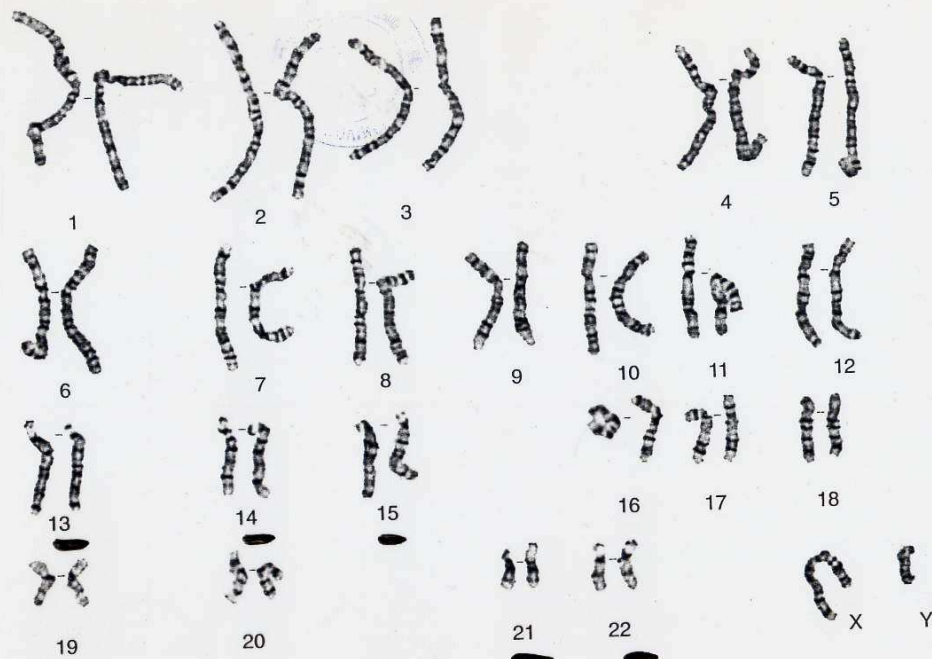
Banda
Regione
Braccio
p
q
1q2.4

CARIOTIPO STANDARD UMANO
(G-banding pattern)
p
q
1
36.2 35 34.2 33 31 21 12 12 22 24 31 41 43
2
24 22 16 14 12 12 14.1 14.3 22 24 32 34 36
3
26 24 22 14 12 13.1 13.3 22 24 26.1 26.3 28
4
15.3 15.1 13 13 22 24 26 28 31.2 32 34
5
15.2 14 12 12 14 21 23 32 34
6
24 22 21.2 12 12 14 16 22 24 26
7
21 14 12 21 31 33 35
8
22 12 12 21.1 21.3 23 24.2
9
23 21 12 12 21 31 33
10
14 12 21 23 25
11
14 12 12 14 22 24
12
12 12 14 21 23 24.2
13
13 21 31 33
14
12 21 23 31
15
12 14 21 23 25
16
13.2 12 11.2 12.2 21 23
17
12 12 22 24
18
11.3 12 22
19
13.2 12 12 13.2 13.4
20
12 12 13.2
21
21
22
12
X
22.2 21 11.3 12 21 23 25 27
Y
11.3 12

Figura 4.3 Cromosomi umani in bandeggio G, alla risoluzione di 550 bande.

ALTA RISOLUZIONE CROMOSOMICA (CROMOSOMI "ALLUNGATI")

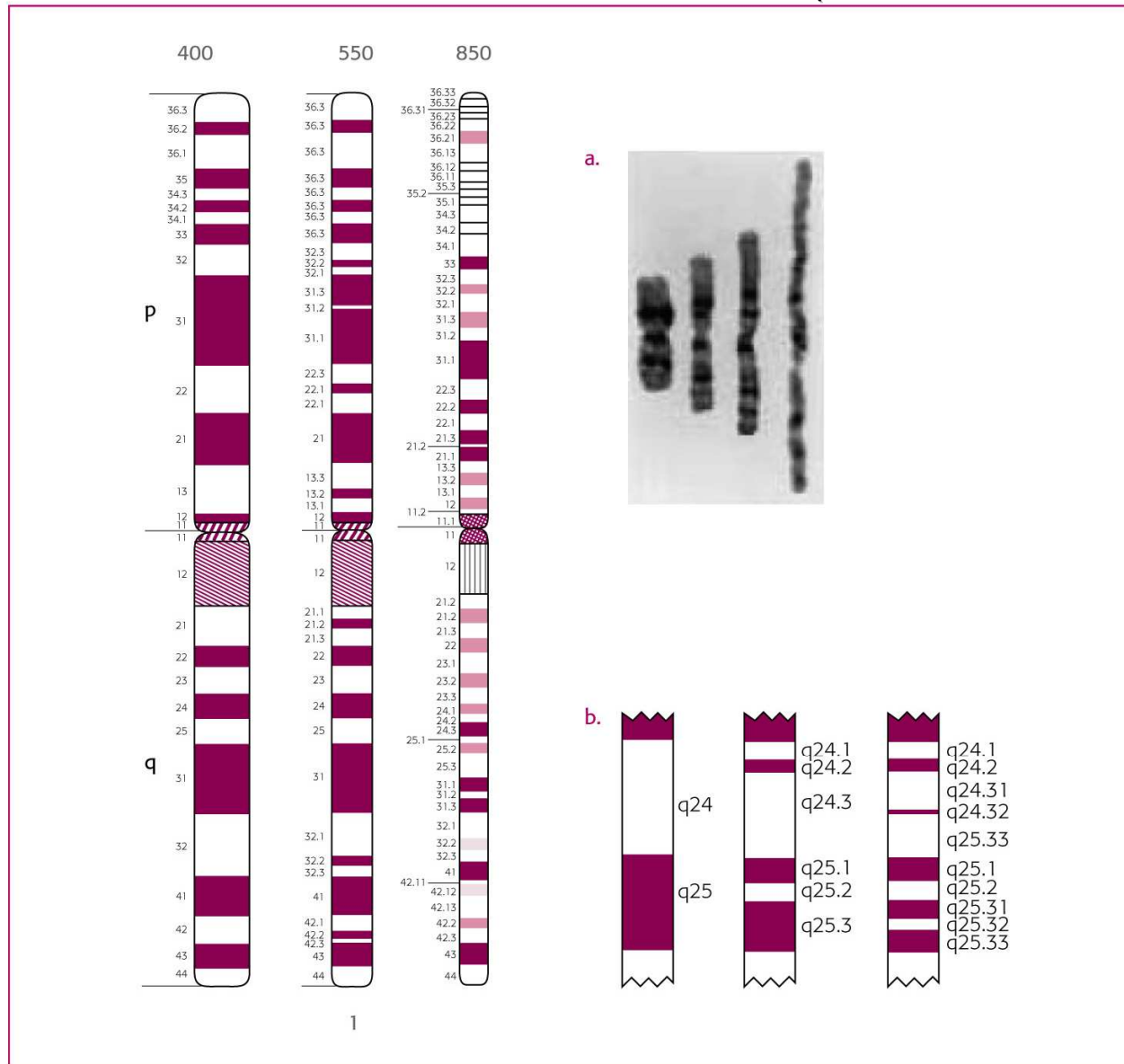
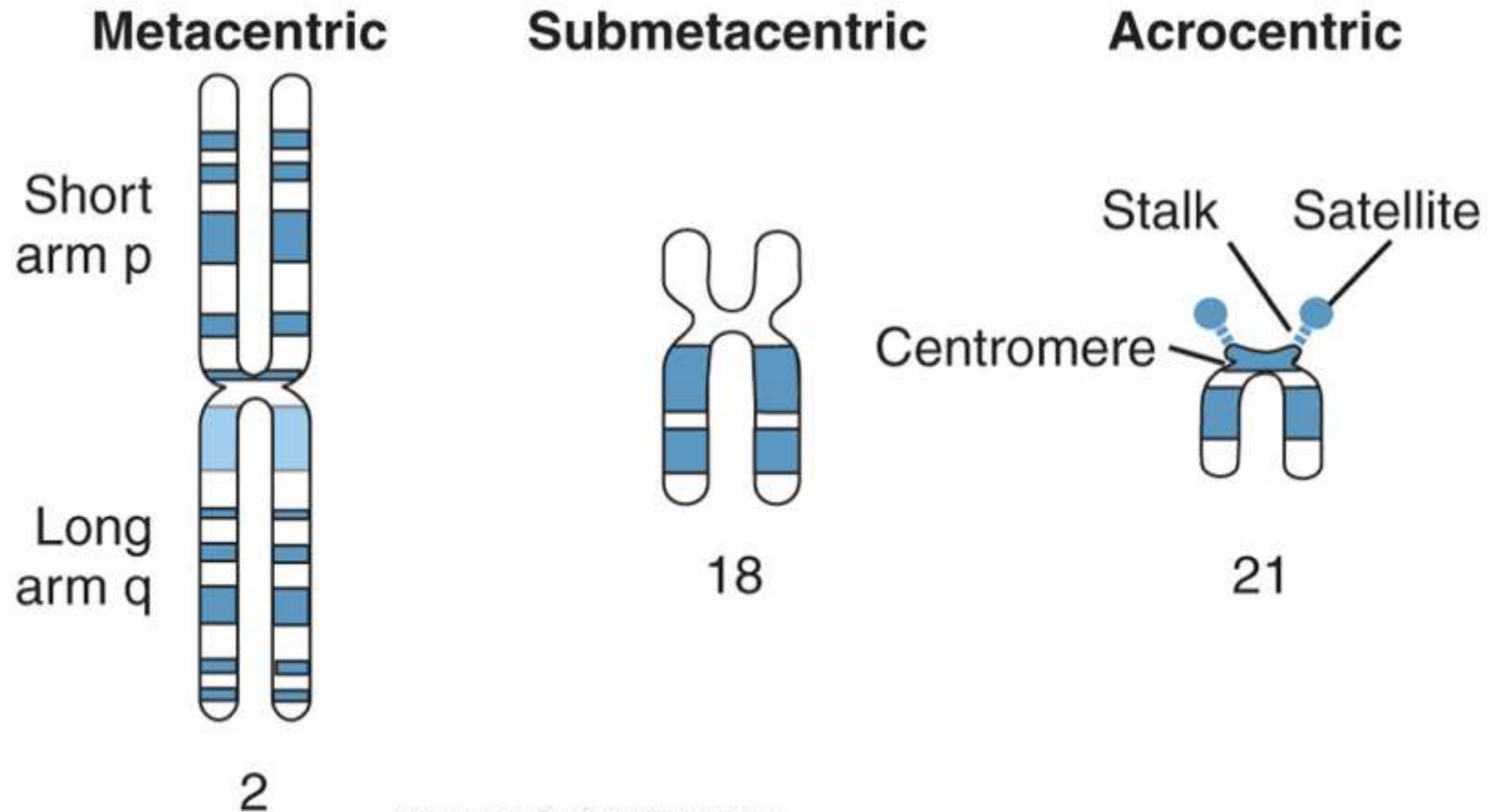



Figura 4.7 A sinistra, idiogramma di bandeggio G di un cromosoma 1 normale a tre diversi livelli di risoluzione (350, 550 e 850 bande per corredo aploide). A destra, cromosoma 1 in bandeggio G a differente risoluzione (a). Numerazione di bande, sottobande e sotto-sottobande (b). (Modificata da Wolstenholme J. *Human Cytogenetics: a practical approach*, vol. 1, 2ª ed. IRL Press, Oxford, 1992.)

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

Morfologia dei cromosomi (dimensioni e posizione centromero)

Jorde et al: Medical Genetics, 4th Edition.

Metacentric, submetacentric, and acrocentric chromosomes. Note the stalks and satellites present on the short arms of the acrocentric chromosomes.

NB Cromosomi acrocentrici

13

14

15

21

22

CITOGENETICA UMANA – NOMENCLATURA STANDARD
(alcuni esempi)

46,XX 46,XY	Normalità
47,XY,+21	Maschio con trisomia 21
46,XX/47,XX,+21	Femmina con mosaicismo
45,XY,t(14:21)	Maschio con traslocazione robertsoniana (NB: t oppure rob oppure der)
46,XX,t(4;5)(p13;q22)	Femmina con traslocazione reciproca
46,XY,inv(2)(p21q23)	Maschio con inversione pericentrica cr.2
46,XX,inv (7) (q11.23q22.1)	Femmina con inversione paracentrica cr.7

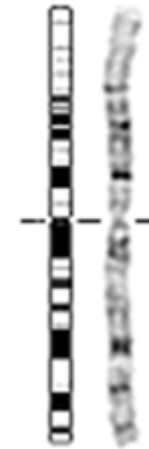
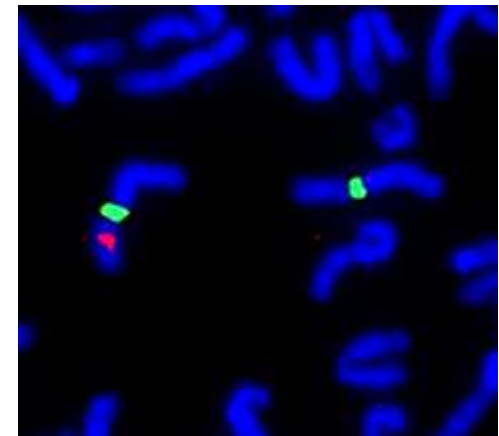
TABLE 6-1

Standard Nomenclature for Chromosome Karyotypes

Karyotype	Description
46,XY	Normal male chromosome constitution
47,XX,+21	Female with trisomy 21, Down syndrome
47,XY,+21[10]/46,XY[10]	Male who is a mosaic of trisomy 21 cells and normal cells (10 cells scored for each karyotype)
46,XY,del(4)(p14)	Male with distal and terminal deletion of the short arm of chromosome 4 from band p14 to terminus
46,XX,dup(5)(p14p15.3)	Female with a duplication within the short arm of chromosome 5 from bands p14 to p15.3
45,XY,der(13;14)(q10;q10)	A male with a balanced Robertsonian translocation of chromosomes 13 and 14. Karyotype shows that one normal 13 and one normal 14 are missing and replaced with a derivative chromosome composed of the long arms of chromosomes 13 and 14
46,XY,t(11;22)(q23;q22)	A male with a balanced reciprocal translocation between chromosomes 11 and 22. The breakpoints are at 11q23 and 22q22
46,XX,inv(3)(p21q13)	An inversion on chromosome 3 that extends from p21 to q13; because it includes the centromere, this is a pericentric inversion
46,X,r(X)(p22.3q28)	A female with one normal X chromosome and one ring X chromosome formed by breakage at bands p22.3 and q28 with subsequent fusion
46,X,i(Xq)	A female with one normal X chromosome and an isochromosome of the long arm of the X chromosome

Jorde et al., 2010

EVOLUZIONE DELLA CITOGENETICA

La Citogenetica Classica (convenzionale) permette di identificare riarrangiamenti cromosomici di non meno di 5 Mb

La Citogenetica Molecolare permette di identificare riarrangiamenti fino a poche Kb

CITOGENETICA MOLECOLARE

Livello di risoluzione< 4-5 Mb

F.I.S.H.

(**F**luorescent **I**n **S**itu **H**ybridization)

Risoluzione: 40Kb-4Mb

Array – CGH

(array-based Comparative Genomic Hybridization)

Risoluzione: 3,5-100Kb/1Mb

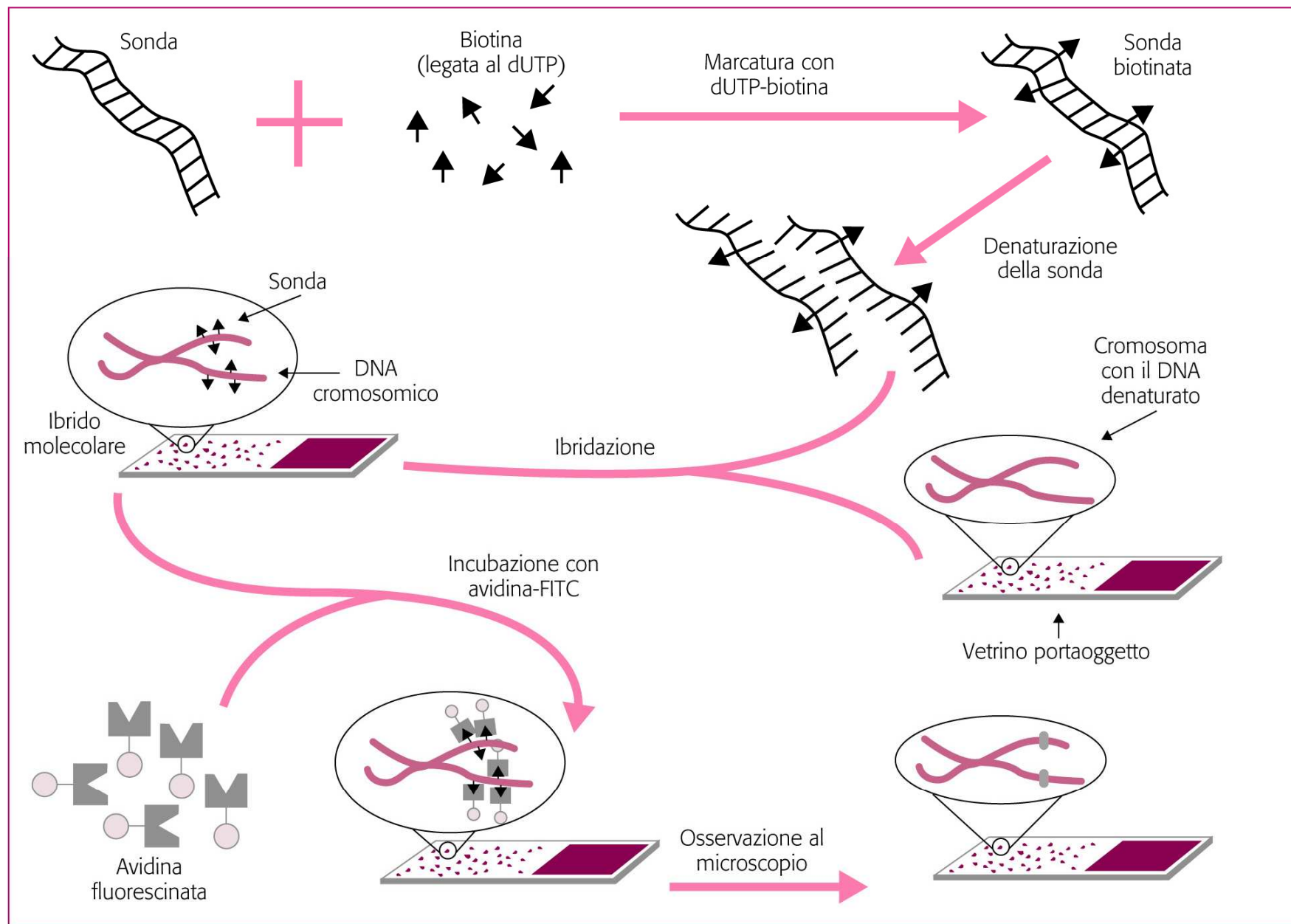
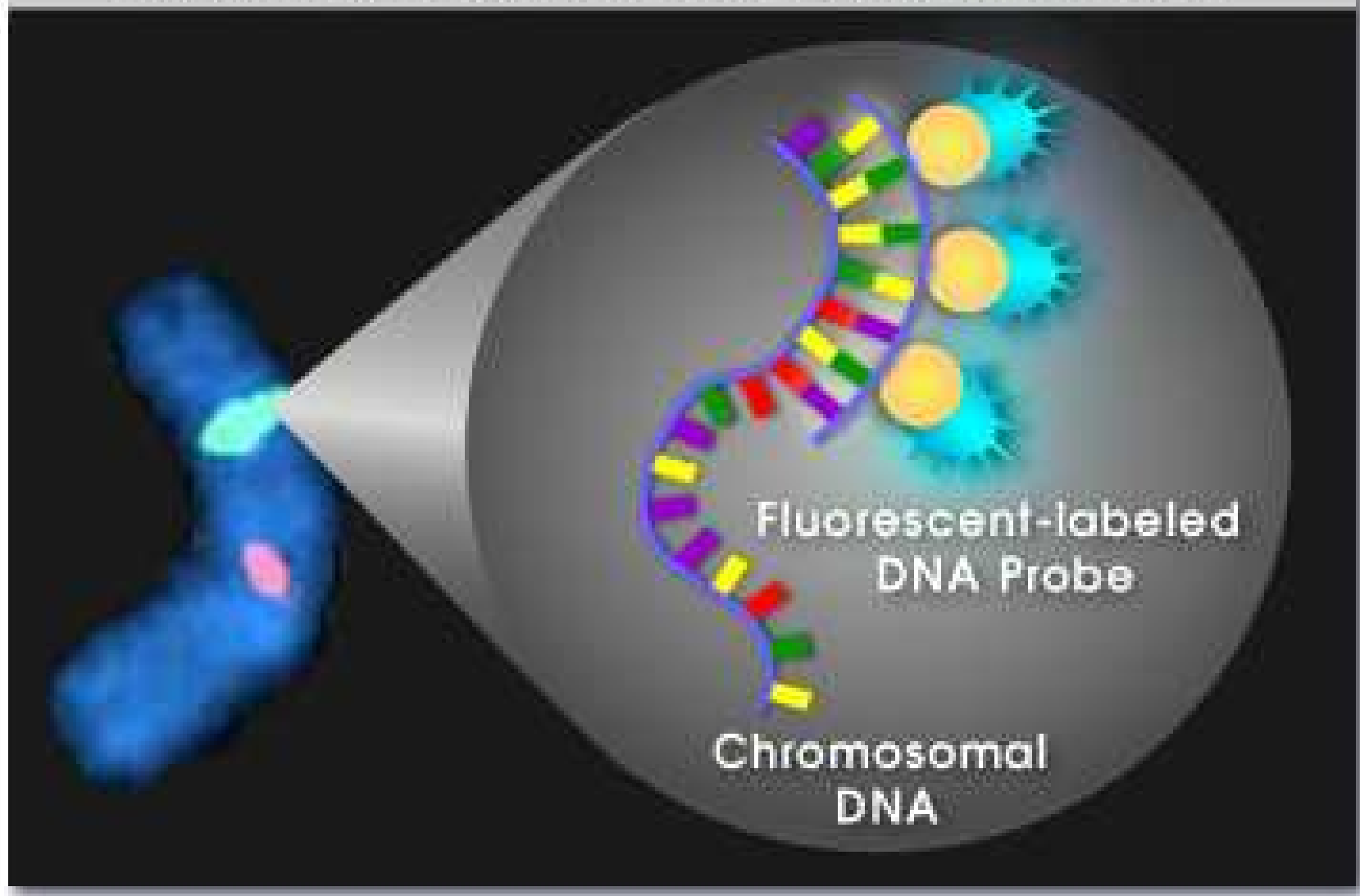
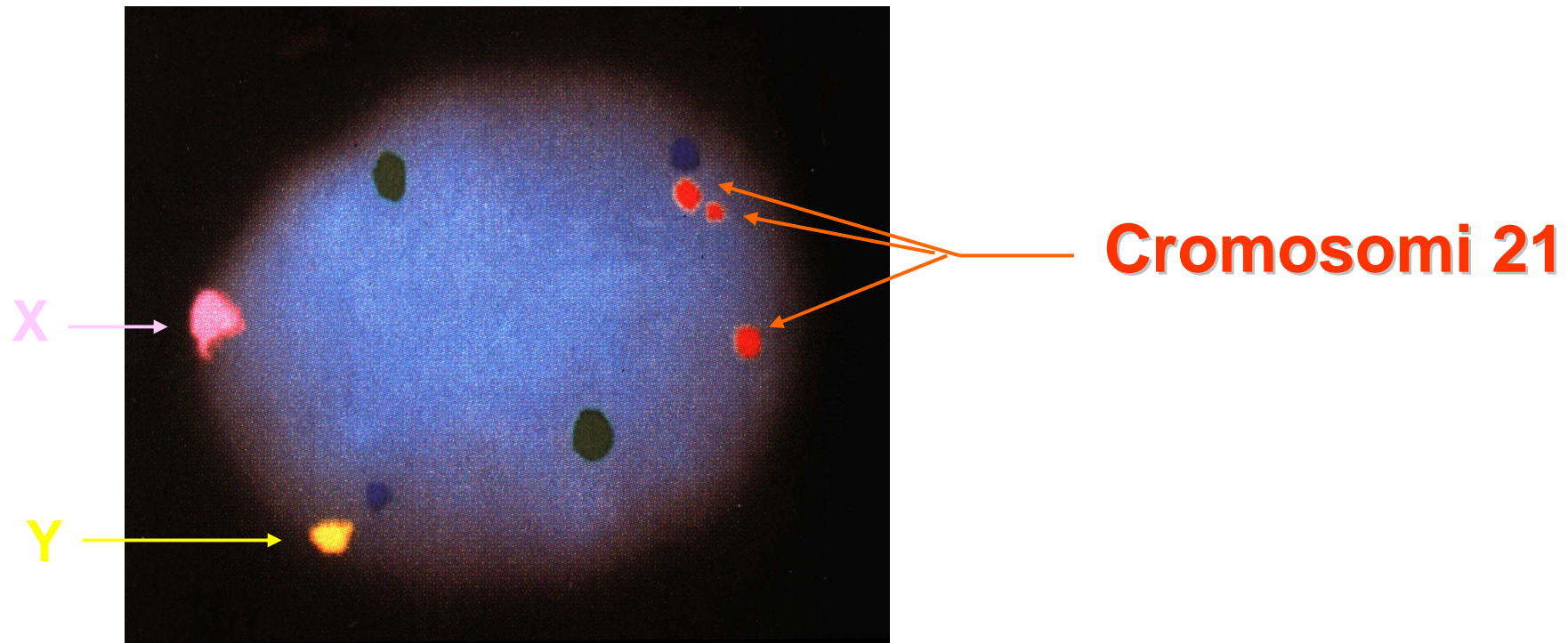
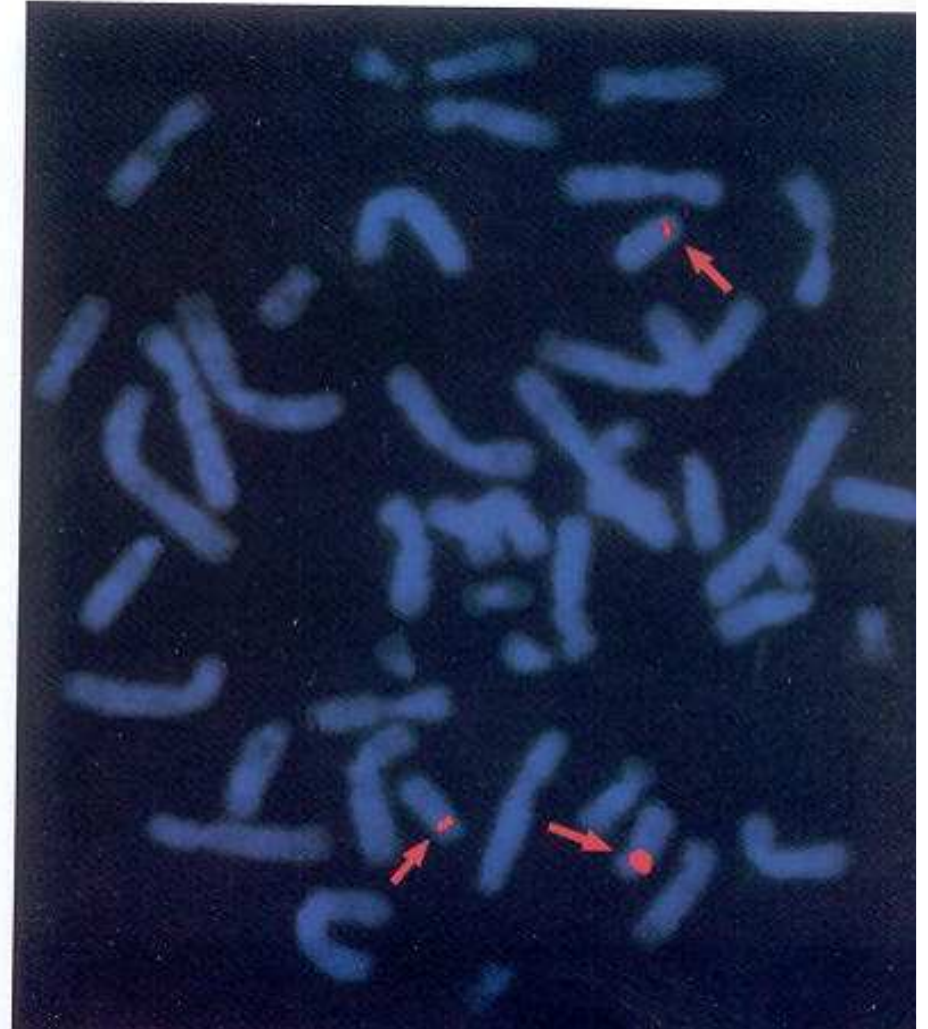
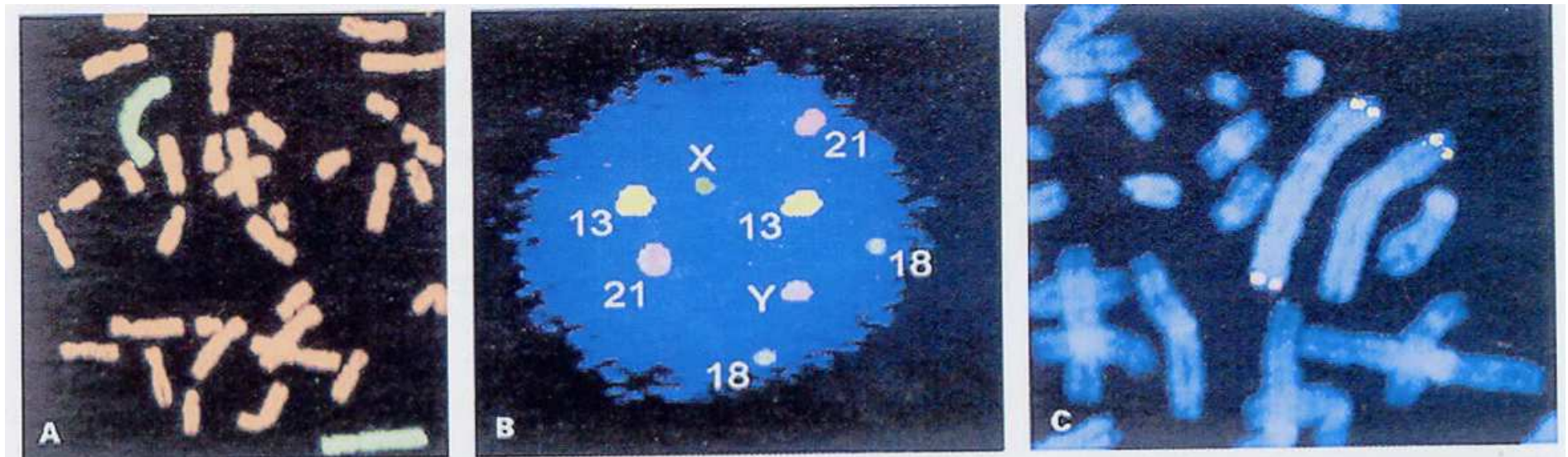



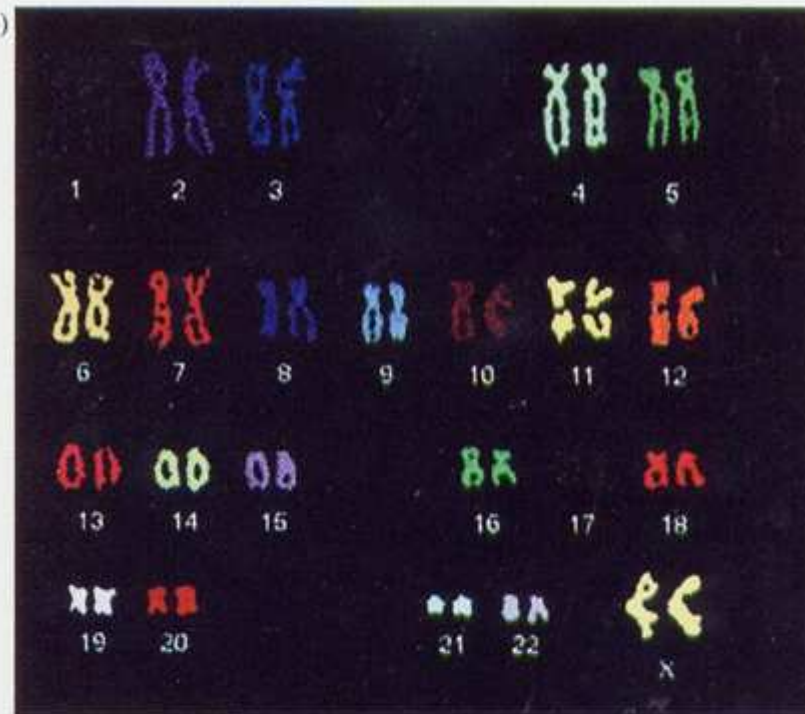
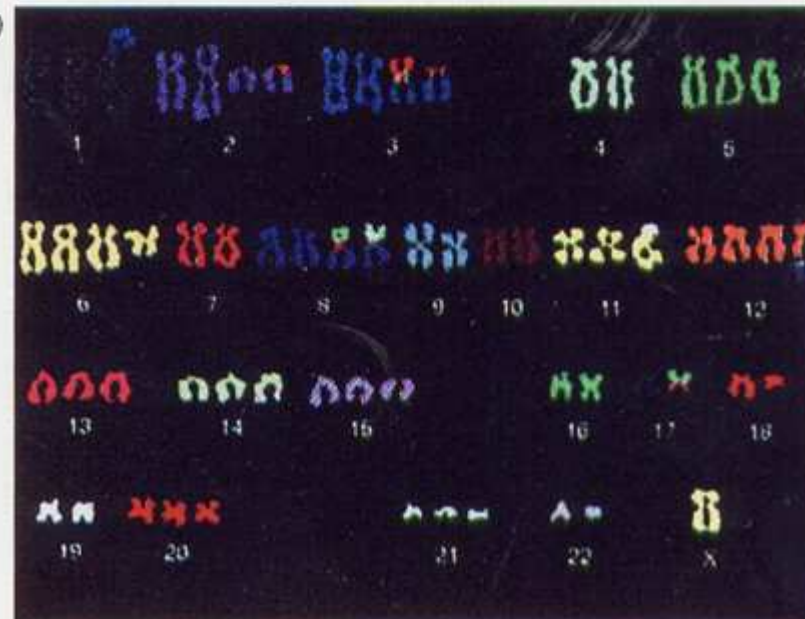
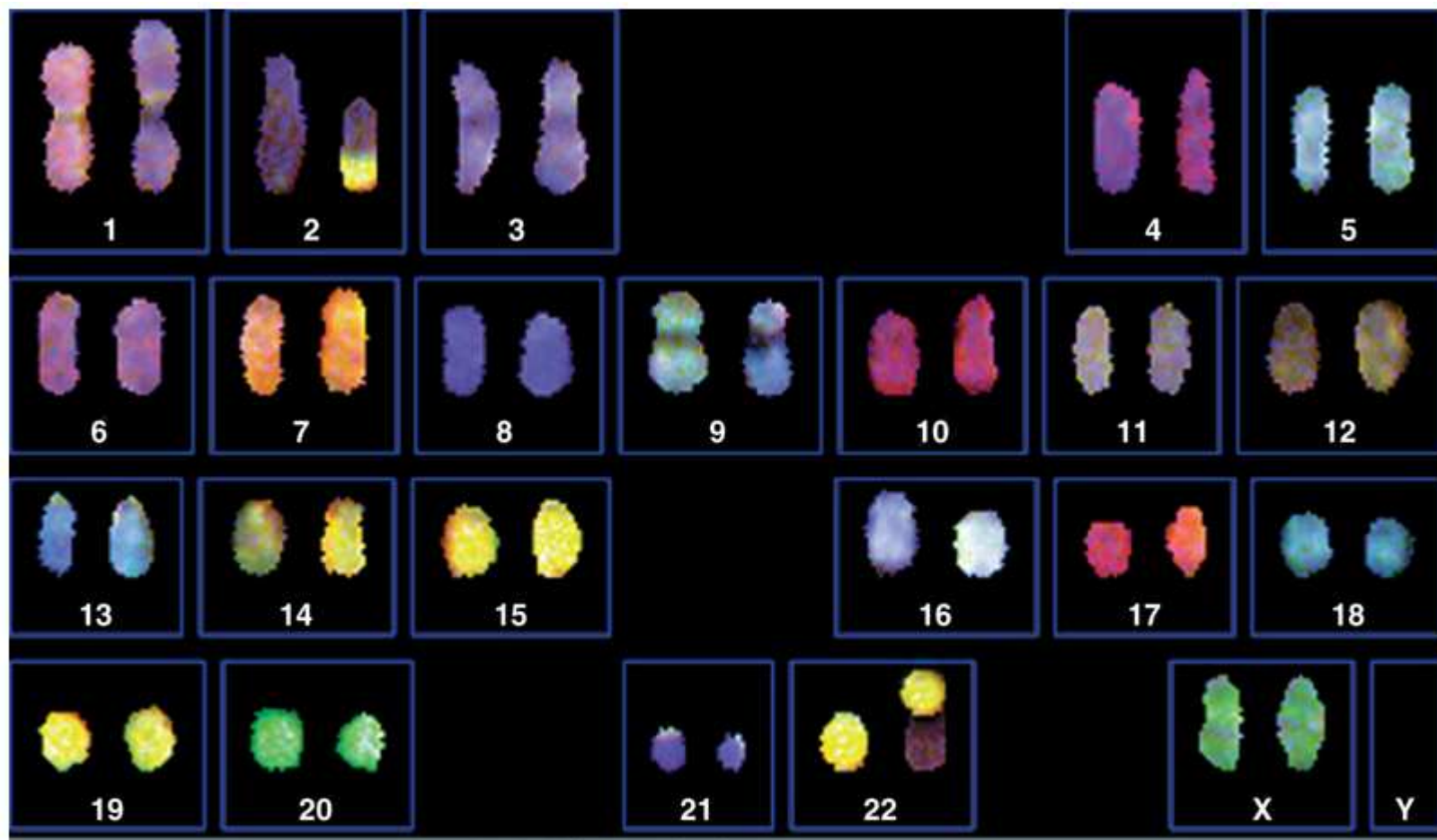
Figura 4.8 Illustrazione esemplificativa delle varie fasi della tecnica FISH. Alcuni passaggi possono essere modificati.

Chromosome prepared using FISH technique
Fluorescent-labeled
DNA Probe
Chromosomal
DNA

FISH

Anni 90, nasce la citogenetica molecolare
Marcatura fluorescente (sonde centromeriche
locus-specifiche, painting)
Elevata risoluzione rispetto a citogen.tradizionale
Rapidità
Nuclei in interfase
Marcature multiple
Elaborazione computerizzata immagini

IBRIDAZIONE FLUORESCENTE "IN SITU" SU NUCLEI IN INTERFASE

Nella foto sono visibili tre macchie rosse corrispondenti a tre cromosomi 21 (s.Down),
2 macchie blu (cr.18), 2 macchie verdi (cr.13), 1 macchia rosa=X, 1 macchia gialla=Y)

Fluorescent In Situ Hybridization

A = painting
B = sonde centromeriche nucleo interfasico
C = sonda telomerica cr.2 (del 2q)

Multicolor FISH
(Painting)

Jorde et al: Medical Genetics, 4th Edition.

Spectral karyotype. The power of spectral karyotyping is demonstrated by the identification of a rearrangement between chromosomes 2 and 22. Note that a portion of chromosome 2 (*purple*) has exchanged places with a portion of chromosome 22 (*yellow*). *(Courtesy of Dr. Art Brothman, University of Utah Health Sciences Center.)*

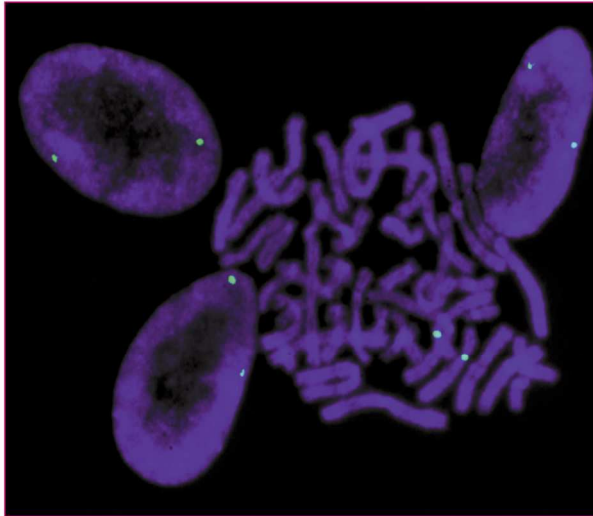



Figura 4.9 Esempio di applicazione della tecnica FISH con sonda centromerica specifica per il cromosoma 8, di cui sono evidenziate due copie. Anche nei nuclei sono presenti due segnali fluorescenti, uno per cromosoma omologo.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

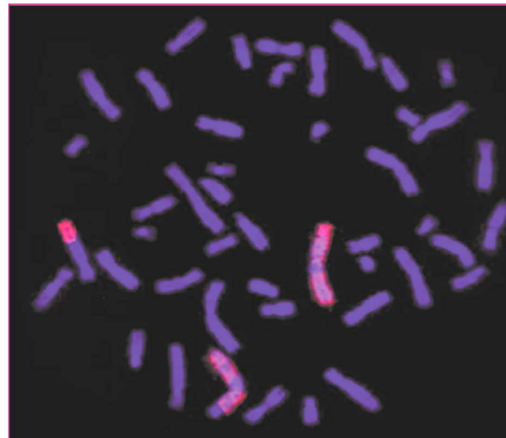
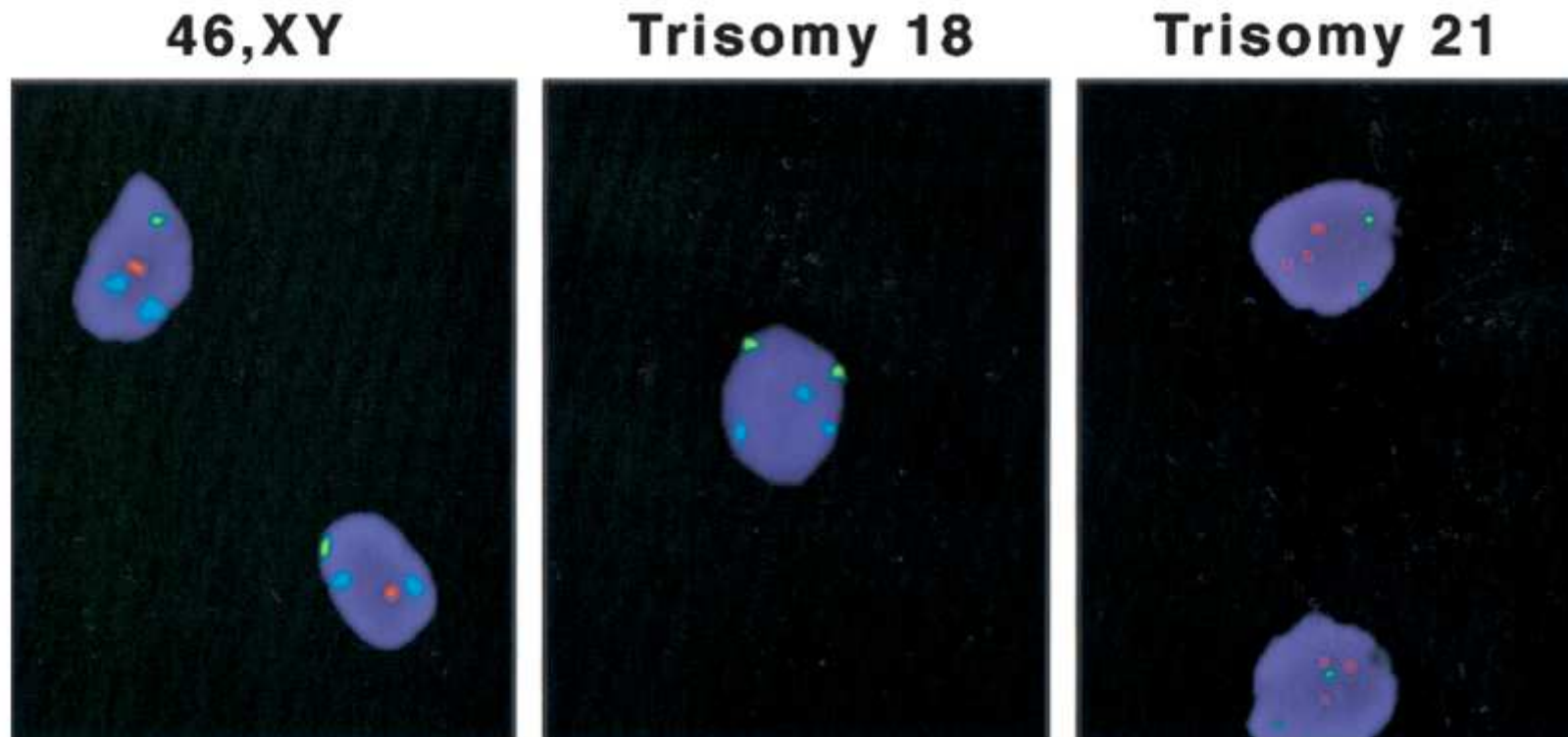



Figura 4.10 Esempio di applicazione della tecnica FISH con sonda painting. Una sonda specifica per il cromosoma 1 ha evidenziato una traslocazione t(1;12).

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

FISH interfasica in diagnosi prenatale

Multicolor fluorescence in situ hybridization analysis of interphase amniotic fluid cells. *Left panel,* 46,XY cells (chromosome 18, *aqua;* X chromosome, *green;* Y chromosome, *red*). *Middle panel,* 47,XX, +18 cell (chromosome 18, *aqua;* X chromosome, *green*). *Right panel,* trisomy 21 cells (chromosome 13, *green;* chromosome 21, *red*). (Courtesy of Stuart Schwartz, University of Chicago.)

P.G.D.

Diagnosi Genetica Preimpianto

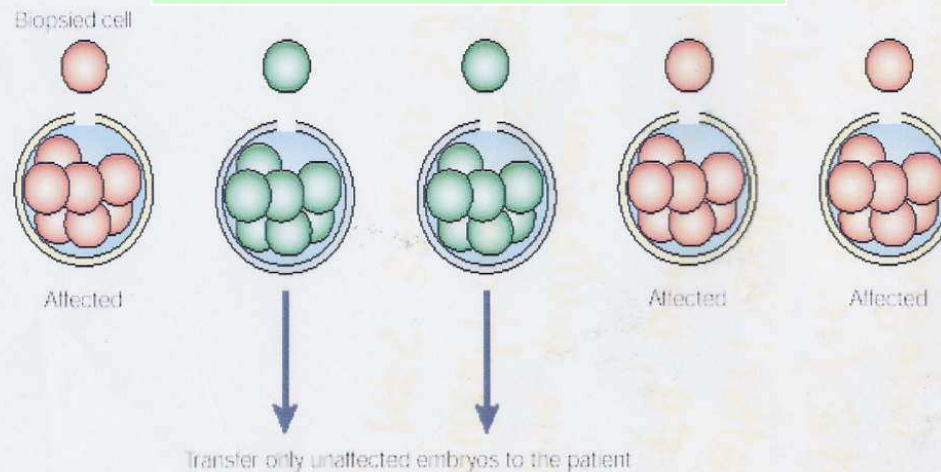



Figure 1 | **Principle of preimplantation genetic diagnosis.** A single cell (or cells) is removed from each embryo of an *in vitro*-developing cohort, on which a diagnostic genetic test is carried out. Up to three of the embryos that are unaffected are transferred to the patient in the hope of establishing a pregnancy

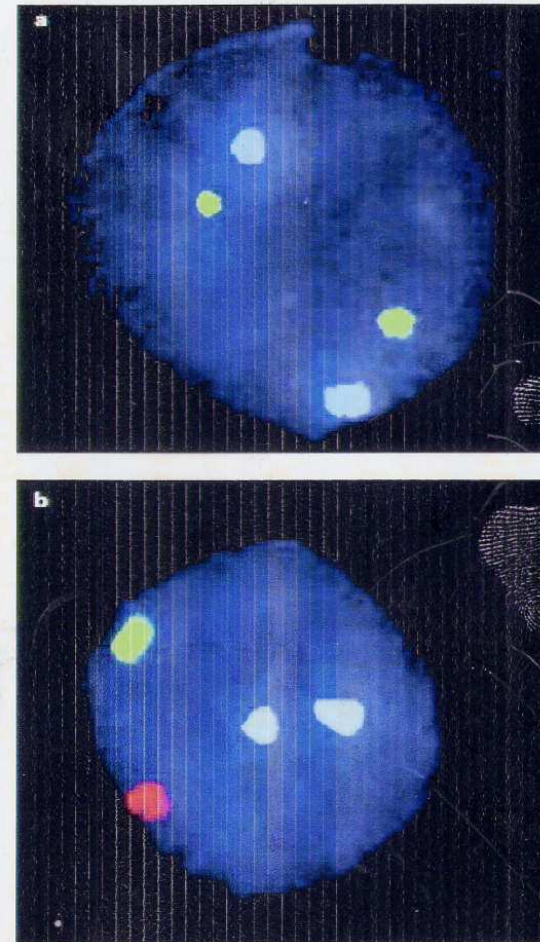



Figure 4 | **PGD of X-linked disorders using FISH.** Two nuclei that have been hybridized with probes that are complementary to sequences on chromosomes X (green), Y (red) and 18 (blue). **a** | A nucleus from the blastomere of a normal female embryo has two green and two blue signals, whereas **b** | a nucleus from a normal male has one red, one green and two blue signals.

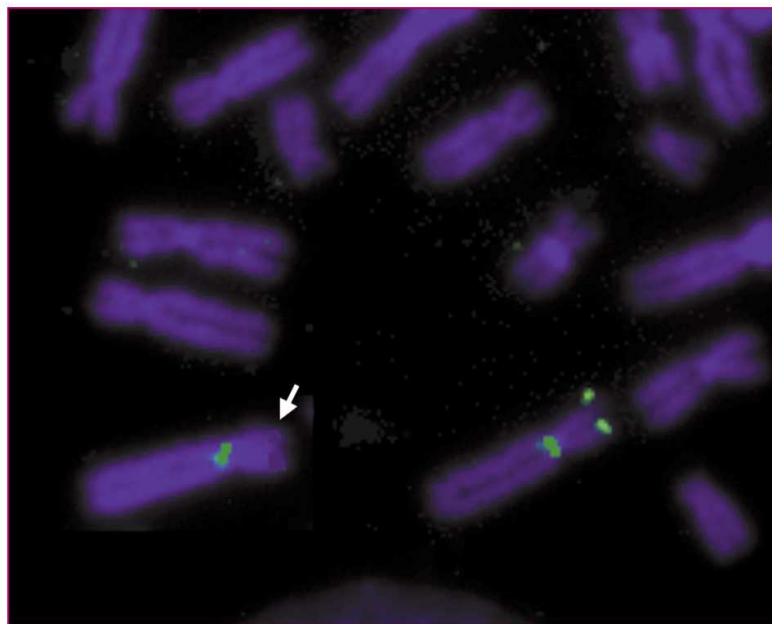



Figura 4.11 Esempio di applicazione della tecnica FISH con sonda *locus*-specifica. In questo caso nel cromosoma 4 normale è presente sia la sonda di repere, che serve a identificare il cromosoma 4 nella metafase, centromerica, sia la sonda subtelomerica specifica per la sindrome di Wolf-Hirschhorn; il cromosoma deleto ha invece solo la sonda centromerica. La delezione osservata è diagnostica per la sindrome di Wolf-Hirschhorn.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

S.Wolf - Hirschhorn

SINDROME DA MONOSOMIA 4p

(Sindrome di Wolf, monosomia 4p)

Grave deficit di accrescimento, ritardo mentale, microcefalia, ipertelorismo, naso grosso e prominente, padiglioni auricolari grandi e poco differenziati.

Figura 13.20 Fenotipo (a) e FISH (b) nella sindrome di Williams-Beuren. Si noti l'assenza della regione critica (in rosso) su uno dei cromosomi 7, riconosciuti dalla sonda verde.

Neri G, Genuardi M. Genetica umana e medica. Elsevier Masson, Milano, 2007

SINDROME DI WILLIAMS

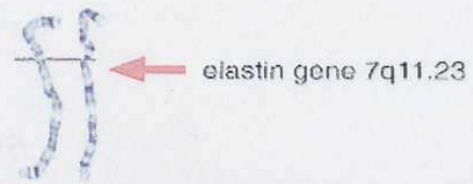
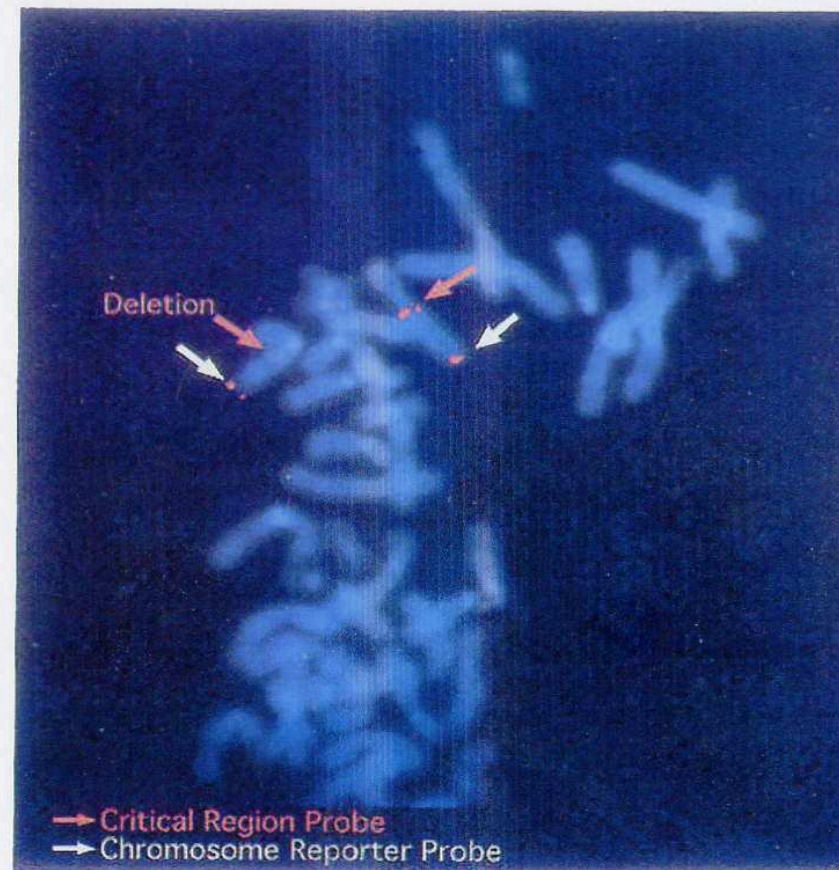
(Elfin face syndrome)

Cardiopatia, guance prominenti, labbra grosse e anteverse.

La s.Williams è causata da una microdelezione cromosomica

Jorde et al: Medical Genetics, 4th Edition.

A, Girl with Williams syndrome, illustrating typical facial features: broad forehead, short palpebral fissures, low nasal bridge, anteverted nostrils, long philtrum, full cheeks, and relatively large mouth with full lips. **B**, Angiogram illustrating supravalvular aortic stenosis (narrowing of the ascending aorta) *(arrow)*. *(Courtesy Dr. Mark Keating, Harvard University.)*

FISH ANALYSIS OF CHROMOSOME 7 - WILLIAMS SYNDROME

Normal

Deletion

CGH, Comparative Genomic Hybridization

FIG. 6-5 The comparative genomic hybridization (CGH) technique. Green-labeled test DNA (from a tumor sample in this case) and red-labeled reference DNA (from normal cells) are both hybridized to normal metaphase chromosomes. The ratio of green to red signal on the hybridized metaphase chromosomes indicates the location of duplications (excess green) and deletions (excess red) in the tumor chromosomes.

ricerca di (micro)delezioni
e/o duplicazioni

FIGURE 3.6 Comparative genomic hybridization of neuroblastoma tumor demonstrating amplification of chromosome 2p23. The middle black line indicates the 1:1 ratio zone of equal mixing of tumor and normal DNA. If the tumor DNA has more of some chromosomal regions than the normal DNA, the distribution line shifts to the right. If the tumor DNA is missing some of the chromosomal regions present in normal DNA, the distribution line shifts to the left. The ratio is displayed in relation to the ISCN ideogram of a metaphase chromosome 2.

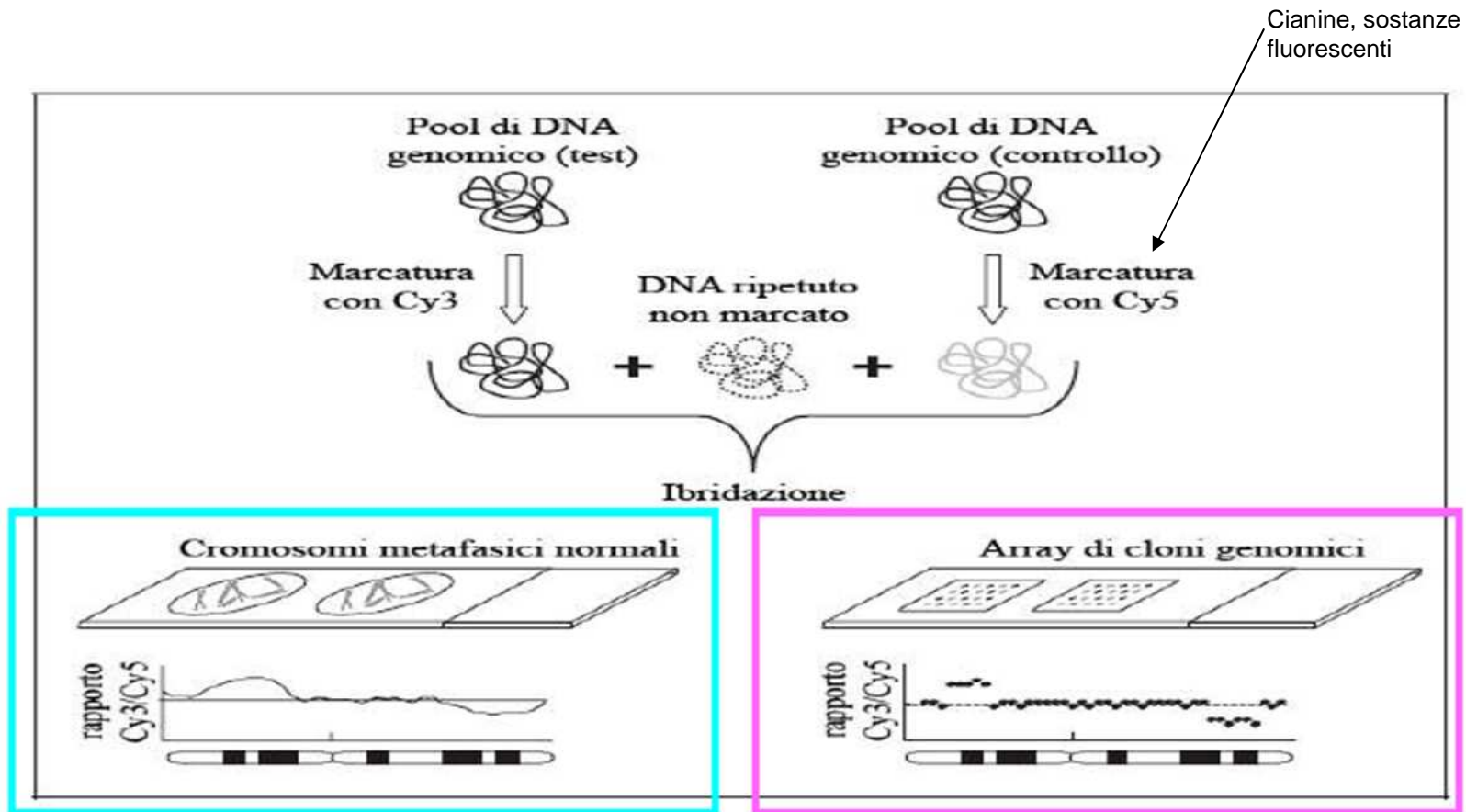
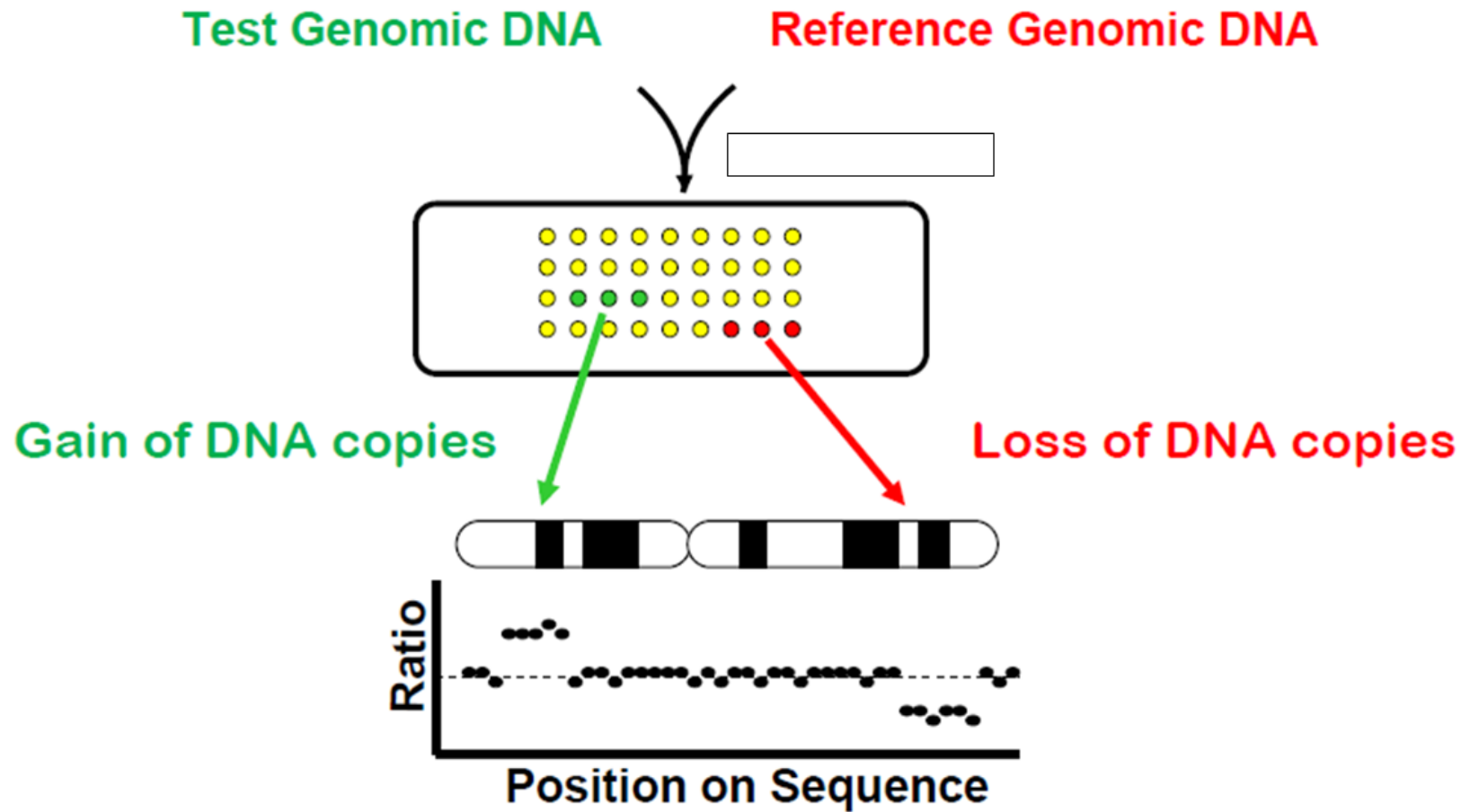
"Medical Genetics for the modern clinicia
J.A.Westman
Lippincott Williams & Wilkins, 2006

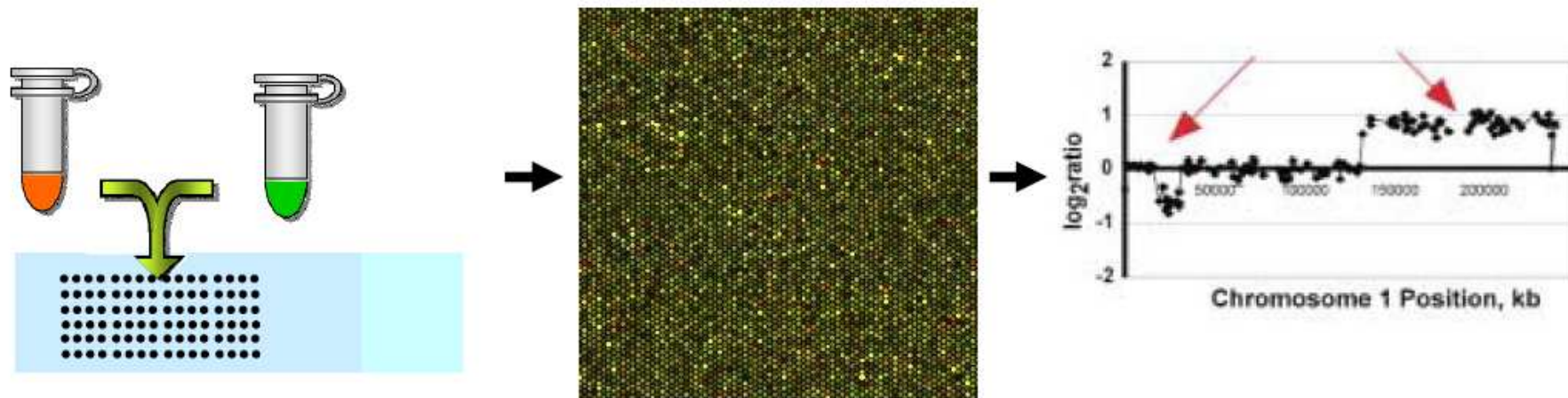
CGH convenzionale e microarray

Screening dell'intero genoma

Cianine, sostanze fluorescenti

Array - CGH

Test Genomic DNA
Reference Genomic DNA
Gain of DNA copies
Loss of DNA copies
Ratio
Position on Sequence

Array-CGH o Molecular Karyotyping

…o CMA (Chromosomal MicroArray)

Vantaggi

- Indipendenza da cellule in divisione
- Capacità di analizzare l'intero genoma in un esperimento
- Elevata specificità, sensibilità e risoluzione
- Rapidità

Identificazione di una microdelezione nel cromosoma 11 (del(11)(q13.2-q13.4) mediante aCGH

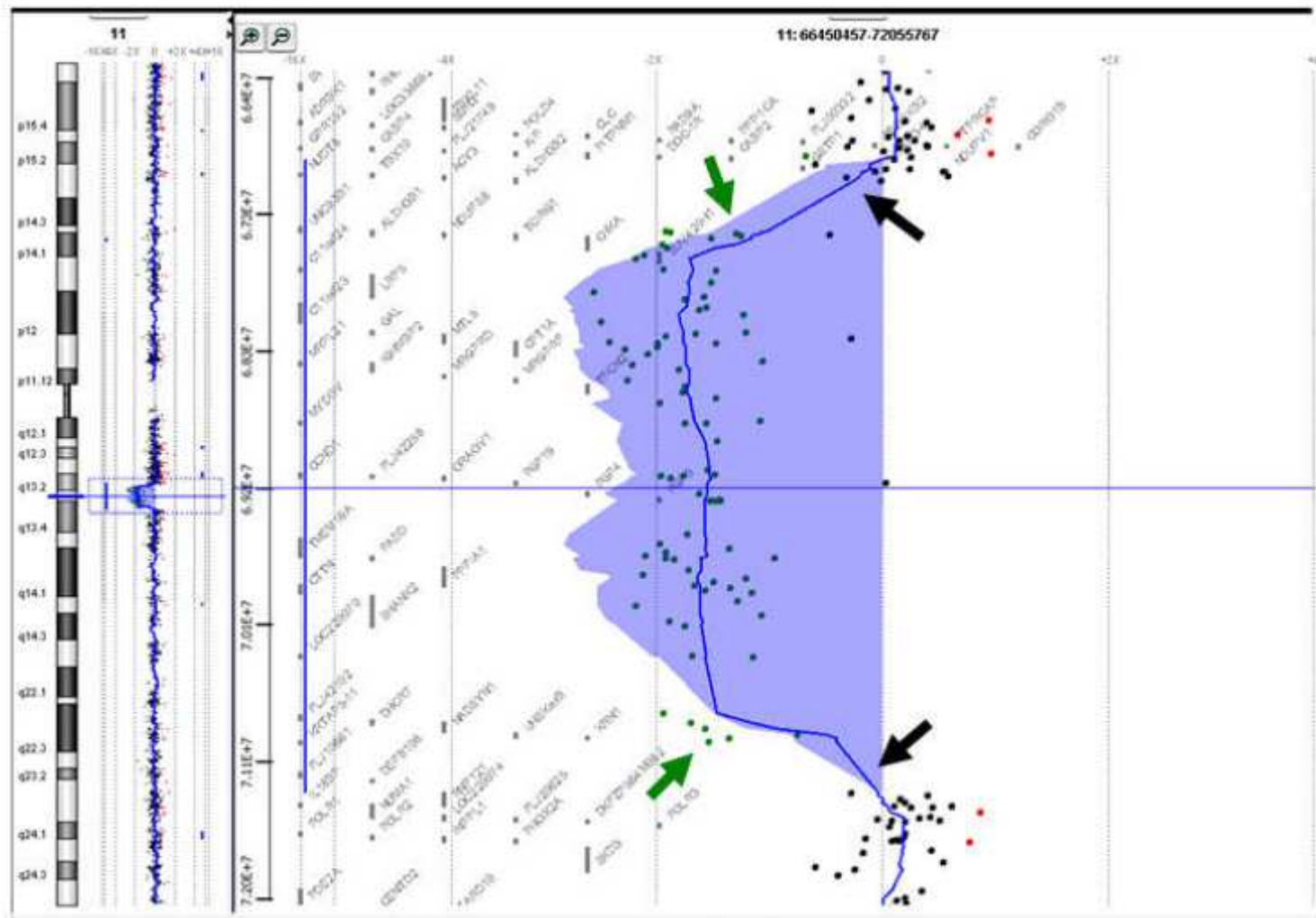
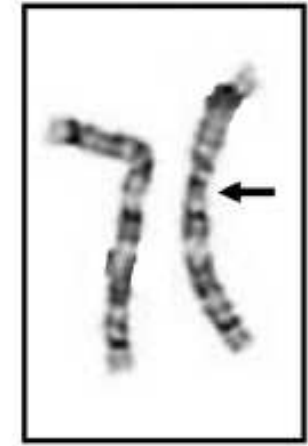
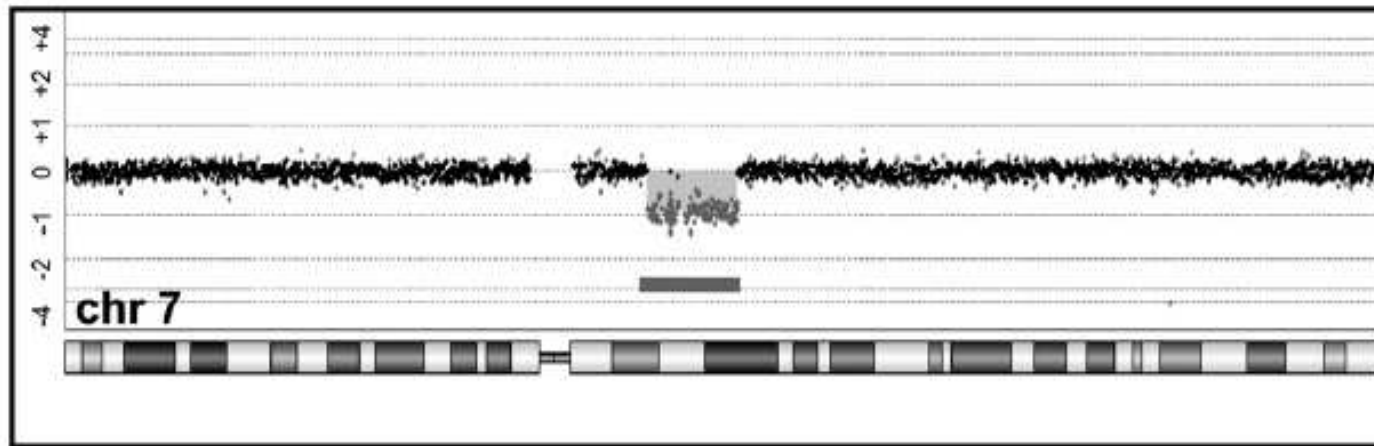
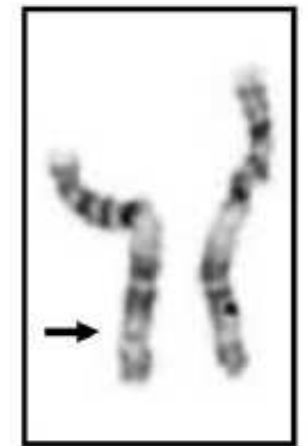
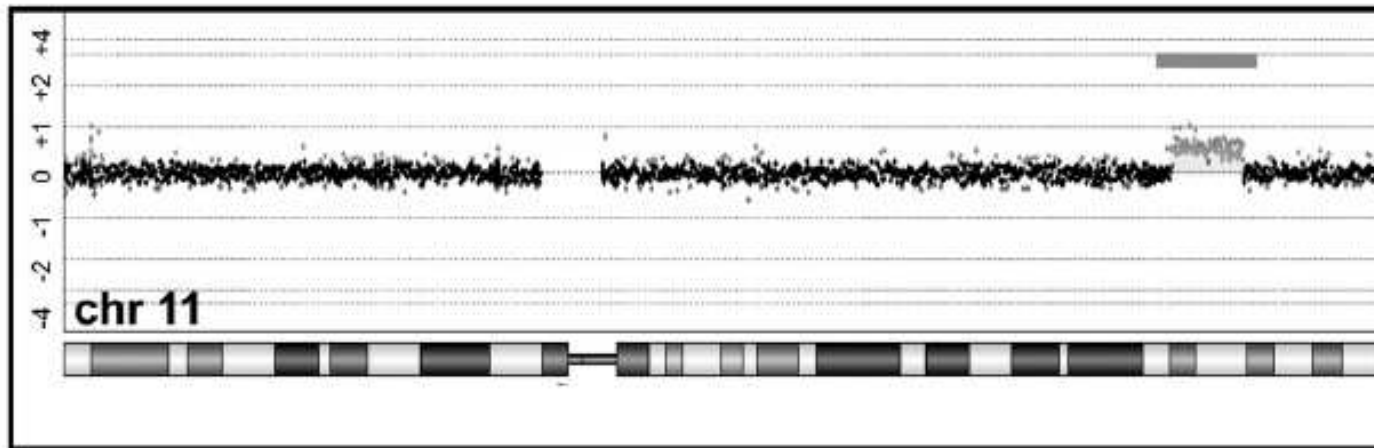
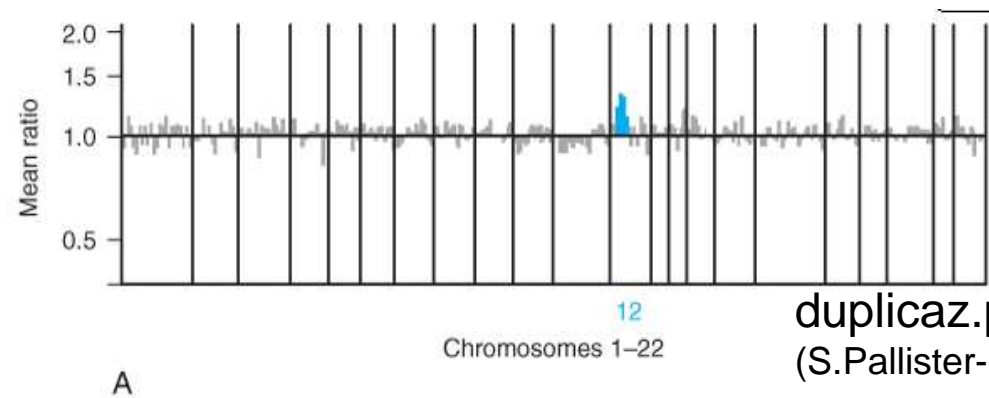
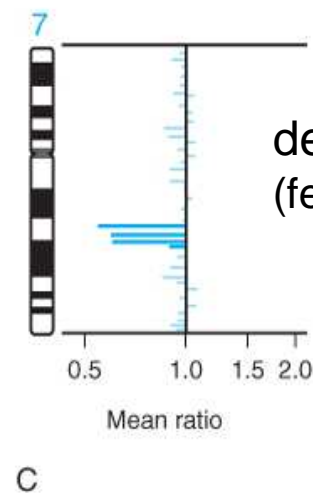
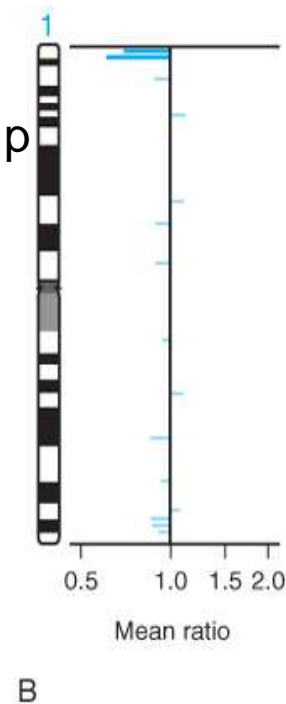



Figure 1. Examples of Genomic Imbalances Detected by a CMA but Not by G-Banded Karyotyping
(A) A 10.9 Mb deletion, including more than 60 genes. The deletion includes the Williams-Beuren syndrome region at chromosome region 7q11 but extends beyond the typical breakpoints for this syndrome. The arrow is pointing to the deleted chromosome that was observed by retrospective analysis of G-banded slides.
(B) A 7.2 Mb duplication on the long arm of chromosome 11. Again, the arrow is pointing to the chromosome that has the duplication shown by the darker G-positive band.

Intensità segnali ibridazione espressa come rapporto (r) su scala log

R = 1 bilanciamento tra campione e controllo

R =1.5 caso:controllo 3:2 (es trisomia)

R = 0.5 caso:controllo 1:2 (es monosomia)

ORIGINAL ARTICLE

Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features

C Shaw-Smith*, R Redon*, L Rickman*, M Rio, L Willatt, H Fiegler, H Firth, D Sanlaville, R Winter, L Colleaux, M Bobrow, N P Carter

See end of article for authors' affiliations

Correspondence to: Dr N P Carter, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; npc@sanger.ac.uk

Revised version received 8 January 2004
Accepted for publication 18 January 2004

J Med Genet 2004;**41**:241–248. doi: 10.1136/jmg.2003.017731

The underlying causes of learning disability and dysmorphic features in many patients remain unidentified despite extensive investigation. Routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than 5 Mb). The presence of subtle DNA copy number changes was investigated by array-CGH in 50 patients with learning disability and dysmorphism, employing a DNA microarray constructed from large insert clones spaced at approximately 1 Mb intervals across the genome. Twelve copy number abnormalities were identified in 12 patients (24% of the total): seven deletions (six apparently de novo and one inherited from a phenotypically normal parent) and five duplications (one de novo and four inherited from phenotypically normal parents). Altered segments ranged in size from those involving a single clone to regions as large as 14 Mb. No recurrent deletion or duplication was identified within this cohort of patients. On the basis of these results, we anticipate that array-CGH will become a routine method of genome-wide screening for imbalanced rearrangements in children with learning disability.

European Journal of Human Genetics (2007) 15, 1105–1114

www.nature.com/ejhg

POLICY

Guidelines for molecular karyotyping in constitutional genetic diagnosis

Joris Robert Vermeesch*[,1], Heike Fiegler[2], Nicole de Leeuw[3], Karoly Szuhai[4], Jacqueline Schoumans[5], Roberto Ciccone[6], Frank Speleman[7], Anita Rauch[8], Jill Clayton-Smith[9], Conny Van Ravenswaaij[10], Damien Sanlaville[11], Philippos C Patsalis[12], Helen Firth[13], Koen Devriendt[1] and Orsetta Zuffardi[6]

[1]*Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium;* [2]*The Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, UK;* [3]*Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands;* [4]*Department of Molecular Cell Biology, Leiden University Medical Centre, Leiden, The Netherlands;* [5]*Department of Molecular Medicine and Surgery, Karolinska Institute, Karolinska University Hospital Solna, Stockholm, Sweden;* [6]*Biologia Generale e Genetica Medica, Università di Pavia, Pavia, Italy;* [7]*Center for Medical Genetics, Ghent University, Ghent, Belgium;* [8]*Institute of Human Genetics, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany;* [9]*Academic Unit of Medical Genetics, St Mary's Hospital, Manchester, UK;* [10]*Department of Genetics, University Medical Center, Groningen, The Netherlands;* [11]*Cytogenetic Department, Hospices Civils de Lyon, Edouard Herriot Hospital, and Claude Bernard University, Lyon, France;* [12]*Department of Cytogenetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus and* [13]*Department of Clinical Genetics, Addenbrooke's Hospital, Cambridge, UK*

Array-based whole genome investigation or molecular karyotyping enables the genome-wide detection of submicroscopic imbalances. Proof-of-principle experiments have demonstrated that molecular karyotyping outperforms conventional karyotyping with regard to detection of chromosomal imbalances. This article identifies areas for which the technology seems matured and areas that require more investigations. Molecular karyotyping should be part of the genetic diagnostic work-up of patients with developmental disorders. For the implementation of the technique for other constitutional indications and in prenatal diagnosis, more research is appropriate. Also, the article aims to provide best practice guidelines for the application of array comparative genomic hybridisation to ensure both technical and clinical quality criteria that will optimise and standardise results and reports in diagnostic laboratories. In short, both the specificity and the sensitivity of the arrays should be evaluated in every laboratory offering the diagnostic test. Internal and external quality control programmes are urgently needed to evaluate and standardise the test results between laboratories.

European Journal of Human Genetics (2007) 15, 1105–1114; doi:10.1038/sj.ejhg.5201896; published online 18 July 2007

The American Journal of Human Genetics 86, 749–764, May 14, 2010

ARTICLE

Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies

David T. Miller,[1,*] Margaret P. Adam,[2,3] Swaroop Aradhya,[4] Leslie G. Biesecker,[5] Arthur R. Brothman,[6] Nigel P. Carter,[7] Deanna M. Church,[8] John A. Crolla,[9] Evan E. Eichler,[10] Charles J. Epstein,[11] W. Andrew Faucett,[2] Lars Feuk,[12] Jan M. Friedman,[13] Ada Hamosh,[14] Laird Jackson,[15] Erin B. Kaminsky,[2] Klaas Kok,[16] Ian D. Krantz,[17] Robert M. Kuhn,[18] Charles Lee,[19] James M. Ostell,[8] Carla Rosenberg,[20] Stephen W. Scherer,[21] Nancy B. Spinner,[17] Dimitri J. Stavropoulos,[22] James H. Tepperberg,[23] Erik C. Thorland,[24] Joris R. Vermeesch,[25] Darrel J. Waggoner,[26] Michael S. Watson,[27] Christa Lese Martin,[2] and David H. Ledbetter[2,*]

Chromosomal microarray (CMA) is increasingly utilized for genetic testing of individuals with unexplained developmental delay/intellectual disability (DD/ID), autism spectrum disorders (ASD), or multiple congenital anomalies (MCA). Performing CMA and G-banded karyotyping on every patient substantially increases the total cost of genetic testing. The International Standard Cytogenomic Array (ISCA) Consortium held two international workshops and conducted a literature review of 33 studies, including 21,698 patients tested by CMA. We provide an evidence-based summary of clinical cytogenetic testing comparing CMA to G-banded karyotyping with respect to technical advantages and limitations, diagnostic yield for various types of chromosomal aberrations, and issues that affect test interpretation. CMA offers a much higher diagnostic yield (15%–20%) for genetic testing of individuals with unexplained DD/ID, ASD, or MCA than a G-banded karyotype (~3%, excluding Down syndrome and other recognizable chromosomal syndromes), primarily because of its higher sensitivity for submicroscopic deletions and duplications. Truly balanced rearrangements and low-level mosaicism are generally not detectable by arrays, but these are relatively infrequent causes of abnormal phenotypes in this population (<1%). Available evidence strongly supports the use of CMA in place of G-banded karyotyping as the first-tier cytogenetic diagnostic test for patients with DD/ID, ASD, or MCA. G-banded karyotype analysis should be reserved for patients with obvious chromosomal syndromes (e.g., Down syndrome), a family history of chromosomal rearrangement, or a history of multiple miscarriages.